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LIVE CELL PRESERVATIVE

[□□□□□□]

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(57) [Abstract]

[Objective]

raffinose is known as component of live cell preservative, but extracting from the natural substance, because it is acquired, also amount of production was limited, also the cost was high.

1 mass production it is possible with microorganism industry - using kestose, it designates that it offers live cell preservative as objective.

[Constitution]

1 - Adjusting kestose concentration 0.1 ~20% (W/V), it makes live cell preservative.

this preservative adding at time of freezing retention or time of the low temperature storage with respect to sperm, or animal cultured cell of sperm, fish of mammal, when you use, is a long term storage effect.

In addition, 1 - kestose being independent, jointly using with other live cell preservative, there is a long term storage effect.

[Claim (s)]

[Claim 1]

live cell preservative. which designates that it contains 1 - kestose as active ingredient as feature

[Claim 2]

live cell preservative. which is stated in Claim 1 which designates that the 1 - kestose concentration was adjusted 0.1 - 20% (W/V) as feature

[Claim 3]

live cell preservative. which is stated in Claim 1 or 2 which designates that it uses for retention of live cell which is chosen from sperm or the animal cultured cell of sperm, fish of mammal as feature

[Description of the Invention]

[0001]

[Field of Industrial Application]

this invention, when long term storage doing live cell with freezing state or low temperature, is sperm of live cell preservative, especially mammals, fish which is necessary and something regarding live cell preservative of animal cultured cell.

[0002]

[Prior Art]

live cell has had life function and biological activity and is a value in use with the polyhedron, but under condition where temperature is high because of the metabolism or modified in time wise change, life function and biological activity decrease or disappear.

It is thought that freezing retention is suited for long term storage of the live cell a this way Japan Unexamined Patent Publication Hei 2- 422 number which uses betaine such as the Japan Unexamined Patent Publication Showa 63-216476 number which uses methylcellulose as for example intracellular frost damage protection quality and Japan Examined Patent Publication Sho 60-29471 number and freezing preservative which assure working effect with extracellular frost damage protection quality and as freezing preservative, many researches redone to today.

[0003]

Generally, you can list following item as condition of substance which shows frost damage preventing effect for live cell.

It must be 1 neutral substance (neutral solute).

They must be 2 low molecular weight substance (Namely, substance where permeability for cell is high).

Have 3 colligative (It is easy to make colligative property, namely hydrogen bond, property where the eutectic point is low).

[0004]

toxicity for cell with as much as 4 high concentration must be low.

At present, in substance which fills up these condition and is widely used, there is a glycerin and a DMSO (dimethylsulfoxide).

But, as for using with alone concerning glycerin being rare, the mixed solution of glycerin and sugar is widely used for sperm freezing retention diluent of animal, for example cattle.

[0005]

As for merit, dilution of one time is possible with room temperature, and it can apply such as you can list freezing rate of wide range where glycerin equilibration time is shortened.

In addition as for frost damage preventing effect of saccharides, 2, from the fact that triose are superior, until recently raffinose which is mainly triose was used in comparison with 5 and 6 charcoal sugar (pentose, hexose).

[0006]

[Problems to be Solved by the Invention]

But, if with impossible, from plant for example beet etc which exists in natural it does not extract and does not refine chemical synthesis of raffinose, it cannot procure.

Because supply amount is limited, it was a expensive.

In addition DMSO, because it is a organic solvent, was a thing where water wash or other operation becomes necessary, handling was complicated generally and those which change to this were strongly desired.

[0007]

This applicant had applied method which produces 1 - kestose of the high purity in inexpensive with sucrose as starting material already and (Japan Patent Application Hei 2- 224312 number), in addition, acquired knowledge of thing which is storage effect of

live cell in this 1- kestose.

this invention designates that live cell preservative which uses 1 - kestose is offered as objective then.

[0008]

[Means to Solve the Problems]

this invention is something which is made live cell preservative which designates that it contains 1 - kestose as active ingredient as feature in order to solve the aforementioned problem.

In addition this invention 1 - is something which is made live cell preservative which designates that 1 - kestose concentration was adjusted 0.1 - 20% (W/V) as feature.

[0009]

In addition this invention is something which is made live cell preservative which designates that 1 - kestose is used for retention of live cell which is chosen from sperm or animal cultured cell of sperm, fish of mammal as feature.

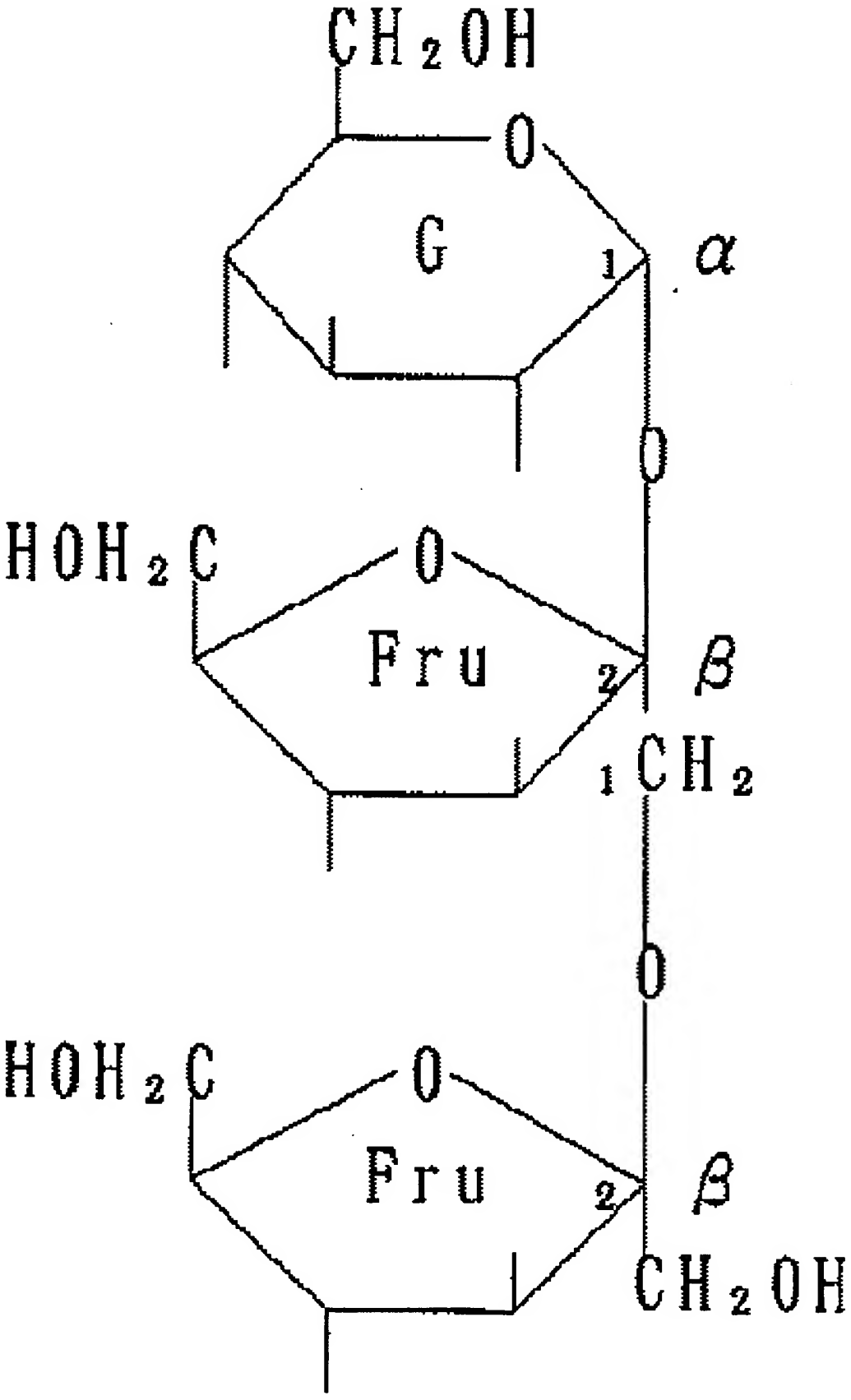
As for 1 - kestose with one kind of so-called fructo-oligo-saccharide, as for structure the glucose (G) with in fructose portion of sucrose (GF) which fructose (F) connects, fructose 1 molecule being something which is connected b- 2, 1 links, it is a O-a-D-gluco-pyranosyl - (1 à 2) -O- b-D- fructo-furanosyl - (1 à 2) -O- b-D- fructo-furanoside.

As for molecular formula, with $C_{18}H_{32}O_{16}$, as for molecular weight 504 is.

1 - structural formula of kestose is shown next.

[0010]

[Chemical Formula 1]


CH2OH
O
G
1
α
O
HOH2C
O
Fru
2
β
1CH2
O
HOH2C
O
Fru
2
β
CH2OH

[0011]

As for application of conventional fructo-oligo-saccharide a bifidus activity and diuretic, etc., which are disclosed in Japan Unexamined Patent Publication Showa 59-110621 number there is, but in each case a mixture, of fructo-oligo-saccharide which are disclosed in Japan Examined Patent Publication Sho 59-53834 number was a mixture of namely 1 kestose (GF_2), nistose (GF_3), fructo-furanosyl-nistose (GF_4).

1 - production method of kestose can be disclosed in Japan Unexamined Patent Publication Hei 2- 163093 number, and the Japan Unexamined Patent Publication Hei 2- 249493 number in sucrose can acquire fructosyl transferase which Scopulariopsis brevicaulis (Scopulariopsis brevicaulis) is produced by fact that it operates.

In addition, this applicant already it applies method which produces 1- kestose of high purity in inexpensive with sucrose as starting material, as the Japan Patent Application Hei 2- 224312 number.

[0012]

With this production method 1 - kestose being single article in industrially, furthermore it is acquired to large scale with high purity (99.9%).

this high purity 1- kestose you obtained knowledge of thing which has possessed effect which is superior as live cell preservative, this invention reached to completion.

Regarding to this invention, 1 you use - you use kestose, with concentration which is suited for live cell which freezing is done, concentration is inside range of 0.1 - 20% (W/V).

In addition you can use 1 - kestose of this invention, as one part or all of the composition of live cell preservative.

At time of freezing retaining of sperm of for example mammal, 0.1 - 0.5%(W/V) you can use as substitution of raffinose which is one composition ones in the existing live cell preservative (sugar content glycerin egg white suspension).

[0013]

In addition, 1 - you can adjust kestose 3 - 20% (W/V) regarding sperm and animal cultured cell of fish, that way you can use with alone as the freezing preservative.

Below, this invention is explained with Working Example, but this is not something where in illustration, this invention is limited to last in this.

[0014]

[Working Example 1]

Concerning freezing retention of sperm of cattle, live cell preservative which includes 1 - kestose of this invention was used, experiment of the storage effect was done.

As control, live cell preservative which consists of composition which includes the raffinose which is a live cell preservative which is used from until recently was used.

In regard to live cell preservative, 1 - kestose with raffinose just substituted things such as control and this invention, other component is all same.

[0015]

composition of freezing storage solution which is used for experiment is shown in the following Table 1.

[0016]

[Table 1]

1. Composition of freezing storage solution for cow sperm
2. Working example 1
3. Reference
4. 2.7% sodium citrate
5. Albumin
6. Tetrose
7. Fructose
8. 1-kestose
9. Glycerin
10. Same as left
11. Same as left
12. Same as left
14. Same as left
15. Raffinose
16. Same as left

牛精子凍結保存液組成

実施例 1	対照	
2.7%クエン酸ナトリウム	同左	76.5 ml
卵白	同左	10.0 ml
デトロース	同左	2 g
フラクトース	同左	1 g
1－ケストース	ラフィノース	0.5 g
グリセリン	同左	10.0 ml

[0017]

experimental condition of freezing retention of sperm of cattle, - with 196 deg C, after between 6 month retaining, thawing doing freezing temperature, inspected sperm vigor.

As for decision of vigor of sperm, you observed sperm with microscope, spirit to be good those which forward are done (+++), those which forward are done (++), those which move head (+) with you did, numeral of sperm vigor (%) with did (+++).

Result is shown in following Table 2.

[0018]

[Table 2]

1. Result of freezing storage solution for cow sperm
2. Breeding bull number
3. Semen volume
4. Sperm count
5. Sperm vigor immediately following collection
6. Sperm vigor after freezing (6 months) and thawing
7. 1-kestose
8. Raffinose

牛精子凍結保存結果

種牛番号	精液量	精子数	採集直後の精子活力	凍結(6カ月)融解後の精子活力	
	ml	10^8/ml		1-ケストース	ラフィノース
H-3026	3.2	9.9	60	30	30
H-3027	3.0	14.1	65	45	45
H-314	7.2	11.0	60	45	50

[0019]

According to Table 2, as for live cell preservative which includes 1 - kestose of this invention, it understands that effect which is equal to live cell preservative which includes conventional raffinose is shown.

[0020]

[Working Example 2]

1 - kestose or aqueous solution, or DMSO solution (Those which are melted in 25 mM Tris-HCl, pH 7.5.) of raffinose 80 μl were added to sperm 20 μl of Oncorhynchus masou (Oncorhynchus masou), final concentration 1 - kestose, as for raffinose as for 60 μM, DMSO solution it becomes 2% requiring, each stock solution was manufactured.

Furthermore, 1 - kestose used those of purity 99.9%.

this way placing each stock solution 50 μl which is

manufactured, in cavity of diameter 5 mm which on dry ice was made with drill, the quick freezing doing, it drew up sperm pellet which freezing is done.

[0021]

- It retained this sperm pellet with 70 deg C, 2 days later and 51 days later thawing did with 4 deg C, inspected motility under microscope.

motility (From start of exercise time to stop) immediately after harvesting/adopting spirit was designated as 100.

Result is shown in following Table 3.

[0022]

[Table 3]

1. Effect of each solution on storage of Oncorhynchus masou sperm
2. 0-th day
3. 2nd day
4. 51st day
5. Solution name
6. Sperm motility
7. Sperm motility
8. Sperm motility
9. Blank
10. Raffinose
11. 1-kestrose

サクラマス精子の保存性に及ぼす各種溶液の効果

溶液名	0日目	2日目	51日目
	精子の運動性	精子の運動性	精子の運動性
ブランク	100	50	40
DMSO	100	80	90
ラフィノース	100	0	0
1-ケストース	100	95	90

[0023]

It understands from Table 3 that it possesses storage effect where the live cell preservative which 1 - kestose of this invention sole use is done is superior.

[0024]

[Working Example 3]

Making use of Hela cell of cancer cell derivation as cultured cell, until with L-15 culture medium which adds 5% fetal calf serum monolayer is formed with 37 deg C the Hela cell which is attached to bottom of flask culture after doing, Hela cell which formed monolayer by digestion doing with the trypsin (EC 3.4.21.4) is peeled.

sterilization fraction collection it did this with pipette which is done, in order to become 3 X 10^5/ml, suspension it did in L-15 culture medium which is a general culture medium for animal cell culture.

1 - kestose, glucose, sucrose, DMSO of 1/5 quantity in order for final concentration to become10%, was added in this.

Furthermore, 1 - kestose used those of purity 99.9%.

This 16 day retention later, room temperature thawing it did with 4 deg C, -20 deg C, -80 deg C and the trypan blue dye after doing, live cell number it measured quantity of live cell with blood cell calculation sheet.

Furthermore, when said dye method is used, core of live cell is dyed, but because it does not dye core of dead cell, survival rate after freezing retaining understands.

Result is shown in following Table 4.

[0025]

[Table 4]

1. Effect of each solution on storage of Hela cells
2. 1-kestrose
3. Glucose
4. Sucrose

Hela細胞の保存性に及ぼす各種溶液の効果

	4℃	−20℃	−80℃
1−ケストース	98	78	92
DMSO	81	93	95
グルコース	96	60	89
シュークロース	95	58	90

[0026]

As for live cell preservative which 1 - kestose of this invention sole use is done in which of 4 deg C, -20 deg C, -80 deg C it understands from Table 4 that storage effect which is superior is shown.

[0027]

[Working Example 4]

With L-15 culture medium which adds 5% fetal calf serum making

use of Vero cell of the kidney cell of African green monkey as cultured cell, with 37 deg C after culture, the digestion it did Vero cell which formed monolayer with trypsin (EC 3.4.21.4), in order to become 1.5 X 10^5/ml, suspension it did in L-15 culture medium.

1 - kestose, glucose, sucrose, DMSO of 1/5 quantity in order for final concentration to become10%, was added in this.

Furthermore, 1 - kestose used those of purity 99.9%.

This 16 day retention later, room temperature thawing it did with 4 deg C, -20 deg C, -80 deg C and the trypan blue dye after doing, live cell number it measured with blood cell calculation sheet.

Result is shown in following Table 5.

[0028]

[Table 5]

1. Effect of each solution on storage of Vero cells
2. 1-kestrose
3. Glucose
4. Sucrose

Vero細胞の保存性に及ぼす各種溶液の効果

	4℃	−20℃	−80℃
1−ケストース	78	53	85
DMSO	76	83	92
グルコース	61	72	53
シュークロース	55	58	78

[0029]

As for live cell preservative which 1 - kestose of this invention sole use is done it understands from Table 5 that storage effect which is superior in 4 deg C, -80 deg C is shown.

Above, this working example was explained on basis of empirical data, but the this invention is not limited in the above-mentioned Working Example, various deformation is possible on basis of gist of this invention.

purity of for example 1- kestose experiment was done concerning 99.9% ones, but it is a usable more even with those of low purity.

[0030]

[Effects of the Invention]

live cell preservative of this invention, 1 - kestose with alone using even when or jointly using with other live cell preservative, has storage effect which is superior anyway even in thing case.

live cell preservative of this invention not only freezing retention, it possesses effect even in retention with low temperature of extent freezing without of doing.

[0031]

1 - Because mass production can do kestose with microorganism industry, live cell preservative can be offered to inexpensive.

PATENT ABSTRACTS OF JAPAN

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(54) LIVE CELL PRESERVATIVE

(57)Abstract:
PURPOSE: To obtain an inexpensive live cell preservative, containing kestose as an active ingredient, having excellent preserving effects and exhibiting effects on not only freezing preservation but also preservation at a low temperature so as not to freeze the live cells.
CONSTITUTION: A live cell preservative containing 1-kestose as an active ingredient, preferably at 0.1-20% (wt./vol.) concentration. The 1-kestose is obtained by reacting a fructosyltransferase produced by Scopulariopsis.brevicaulis with, e.g. sucrose.

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CLAIMS

[Claim(s)]
[Claim 1] The viable cell preservative characterized by containing 1-kestose as an active principle.
[Claim 2] The viable cell preservative according to claim 1 characterized by adjusting 1-kestose concentration to 0.1 - 20% (W/V).
[Claim 3] The viable cell preservative according to claim 1 or 2 characterized by using for preservation of the viable cell chosen from the sperm of mammalian, the sperm of fishes, or the animal culture tissue.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] In case this invention saves a viable cell at a freezing condition or low temperature for a long period of time, it relates to the sperm of a required viable cell preservative especially the mammals, and fishes, and the viable cell preservative of an animal culture tissue.
[0002]
[Description of the Prior Art] Although the viable cell has a living function and biological activity, and is various and there is utility value, under the conditions that temperature is high, change, a living function, and biological activity will fall thru/or disappear with time because of a metabolic turnover or denaturation. Many researches are done till today, such as JP,60-29471,B which was considered that cryopreservation is suitable for the mothball of such a viable cell, for example, planned the having-two-incomes effectiveness of an intracellular frost damage protector and an extracellular frost damage protector, JP,63-216476,A which used methyl cellulose as a cryoprotective agent, and JP,2-422,A which used the betaine as a cryoprotective agent.
[0003] Generally, the following item is mentioned as conditions for the matter which show the frost damage prevention effectiveness over a viable cell.
** It is the neutral matter (neutral solute).
** It is the low-molecular matter (namely, matter with the high permeability over a cell).
** Have colligative property (it is easy to make colligative property, i.e., hydrogen bond, and the eutectic point is a low property).
[0004] ** The high concentration of the toxicity over a cell is also low.
A glycerol and DMSO (dimethylsulfoxide) are in the matter which fulfills these conditions and is now used widely. However, it is rare to use it independently about a glycerol, and the mixed liquor of a glycerol and sugar is widely used for the sperm cryopreservation diluent of an animal, for example, a cow.
[0005] It is raised that that one-time dilution is possible for the merit at a room temperature, a glycerol balancing time's being shortened, and the freezing velocity of the large range are applicable etc. Moreover, since the frost damage prevention effectiveness of a saccharide excels 5 and 6 **** (Pentose, Hexose) in 2 and 3 saccharide, the raffinose which are mainly three saccharides has been used conventionally.

[0006]
[Problem(s) to be Solved by the Invention] However, the chemosynthesis of a raffinose is impossible, and if it does not extract and refine, the vegetation, for example, the beat etc., etc. which exists naturally, it cannot come to hand. Since the amount of supply was restricted, it was expensive. Moreover, since DMSO was an organic solvent, actuation of washing in cold water etc. might be needed, generally handling was complicated and what changes to this was desired strongly.
[0007] These people acquired the knowledge of it having already applied for the approach of manufacturing 1-kestose of a high grade cheaply by using cane sugar as a raw material (Japanese Patent Application No. No. 224312 [two to]), and the preservation effectiveness of a viable cell being in this 1-kestose. Then, this invention aims at offering the viable cell preservative which used 1-kestose.
[0008]
[Means for Solving the Problem] In order to solve said trouble, let this invention be the viable cell preservative characterized by containing 1-kestose as an active principle. Moreover, let this invention be the viable cell preservative characterized by adjusting 1-kestose concentration to 0.1 - 20% (W/V).
[0009] Moreover, let this invention be the viable cell preservative characterized by using 1-kestose for preservation of the viable cell chosen from the sperm of mammalian, the sperm of fishes, or the animal culture tissue. 1-kestose is so-called kind of a fructo oligosaccharide, fructose carries out 1 molecular binding of the structure to the fructose part of the shoe cloth (GF) which a glucose (G) and fructose (F) combined by beta-2 and 1 association, and it is O-alpha-D-glucopyranosyl. -(1->2)- It is an O-beta-D-hula KUTOFU llano sill. -(1->2)- It is O-beta-D-FURAKUTO furanoside. The molecular formula is C18H32O16, and the molecular weight is 504. Next, the structure expression of 1-kestose is shown.
[0010]
[Formula 1]

[0011] Although the application of the conventional fructo oligosaccharide had the diuretic currently indicated by the BIFIZUSU activity currently indicated by JP,59-53834,B and JP,59-110621,A, all were the mixture of the mixture of a fructo oligosaccharide, i.e., 1 kestose, (GF2), nistose (GF3), and FURAKUTO furanosyl nistose (GF4). The manufacturing method of 1-kestose is indicated by JP,2-163093,A and JP,2-249493,A, and can be acquired by making the cell tosyl transferase which the Scopulariopsis BUREBI cow squirrel (Scopulariopsis brevicaulis) produces act on cane sugar. Moreover, these people have already applied for the approach of manufacturing 1-kestose of a high grade cheaply by using cane sugar as a raw material, as Japanese Patent Application No. No. 224312 [two to].
[0012] According to this producing method, moreover, 1-kestose is industrially obtained in large quantities by the high grade (99.9%) individually. This high grade 1-kestose acquires the knowledge of having the effectiveness which was excellent as a viable cell preservative, and came to complete this invention. Using 1-kestose used in this invention by the concentration suitable for the viable cell to freeze, the concentration is 0.1 - 20% (W/V) of within the limits. Moreover, 1-kestose of this invention can be used as a part or all of a presentation of a viable cell preservative. For example, in the cryopreservation of the sperm of mammalian, use can be carried out 0.1 to 0.5% (W/V) as an alternative of the raffinose which is one constituent in the existing viable cell preservative (a sugar content glycerol and albumen suspension).
[0013] Moreover, in the sperm and animal culture tissue of fishes, 1-kestose can be adjusted to 3 - 20% (W/V), and it can be independently used as a cryopreservation agent as it is. Although it has an example in below and this invention is explained to it, this is instantiation to the last and this invention is not limited to this.
[0014]
[Example 1] About the cryopreservation of the sperm of a cow, the viable cell preservative containing

1-kestose of this invention was used, and it experimented in the preservation effectiveness. The viable cell preservative which consists of a constituent containing the raffinose which is the viable cell preservative currently used from the former as contrast was used. About the viable cell preservative, the raffinose only replaced the thing of contrast and this invention with 1-kestose, and all other components are the same.
[0015] The presentation of the cryopreservation liquid used for the experiment is shown in the next table 1.
[0016]
[Table 1]

[0017] After the experiment conditions of the cryopreservation of the sperm of a cow are -196 degrees C and saved freezing temperature for six months, they were thawed and investigated motility of sperm. The judgment of the vital force of a sperm should observe the sperm under the microscope, should move what moves forward with sufficient vigor (+++), the thing (++) which moves forward, and the head (+), and made (+++) the figure (%) of motility of sperm. The result is shown in the next table 2.
[0018]
[Table 2]

[0019] According to Table 2, it turns out that the viable cell preservative containing 1-kestose of this invention shows effectiveness equivalent to the viable cell preservative containing the conventional raffinose.
[0020]
[Example 2] 1-kestose, the water solution of a raffinose, or the DMSO solution (what was dissolved in 25mM Tris-HCl and pH7.5) was 80microl Added to 20micro of sperms l of a cherry salmon (Oncorhynchus masou), and 60microM and a DMSO solution prepared each stock solution, as 1-kestose and a raffinose became 2% about the last concentration. In addition, 1-kestose used the thing of 99.9% of purity. Thus, it put on the hollow with a diameter of 5mm which made 50micro of each prepared stock solution l with the drill on dry ice, and it quick-froze and the frozen sperm pellet was created.
[0021] This sperm pellet was saved at -70 degrees C, it thawed at 4 degrees C two days and 51 days after, and maneuverability was investigated under the microscope. Maneuverability just behind **** (time amount from movement initiation to a halt) was set to 100. The result is shown in the next table 3.
[0022]
[Table 3]

[0023] Table 3 shows having the preservation effectiveness excellent in the viable cell preservative which carried out independent use of the 1-kestose of this invention.
[0024]
[Example 3] After cultivating until it forms a monolayer at 37 degrees C by L-15 culture medium which added fetal calf serum 5%, using the Hela cell of the gun cell origin as a cultured cell, the Hela cell attached to the bottom of a flask is removed by digesting the Hela cell in which the monolayer was formed, by the trypsin. It isolated preparatively with the pipet which sterilized this and suspended in L-15 culture medium which is a general culture medium for animal cell culture so that it may be set to 3x105 / ml. 1-kestose of 1/5 amount, a glucose, shoe cloth, and DMSO were added to this so that the last concentration might become at 10%. In addition, 1-kestose used the thing of 99.9% of purity. After carrying out the room temperature defrosting of this after preservation for 16 days at 4 degrees C, -20 degrees C, and -80 degrees C and carrying out trypan blue dyeing of the viable count, the number of viable cells was measured by the counting chamber. In addition, if this staining technique is used, the

nucleus of a viable cell will be dyed, but since the nucleus of a dead cell is not dyed, the survival rate after cryopreservation is known. The result is shown in the next table 4.
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[0026] It turns out that the viable cell preservative which carried out independent use of the 1-kestose of this invention from Table 4 shows the preservation effectiveness which was excellent also in any (4 degrees C, -20 degrees C, and -80 degrees C).
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[Example 4] The Vero cell which formed the monolayer after culture at 37 degrees C was digested by the trypsin in L-15 culture medium which added fetal calf serum 5%, using the Vero cell of the nephrocyte of an African green monkey as a cultured cell, and it suspended in L-15 culture medium so that it might be set to 1.5x105 / ml. 1-kestose of 1/5 amount, a glucose, shoe cloth, and DMSO were added to this so that the last concentration might become at 10%. In addition, 1-kestose used the thing of 99.9% of purity. After carrying out the room temperature defrosting of this after preservation for 16 days at 4 degrees C, -20 degrees C, and -80 degrees C and carrying out trypan blue dyeing of the viable count, it measured by the counting chamber. The result is shown in the next table 5.
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[0029] It turns out that the viable cell preservative which carried out independent use of the 1-kestose of this invention from Table 5 shows the preservation effectiveness excellent in 4 degrees C and -80 degrees C. As mentioned above, although this example was explained based on experimental data, this invention is not limited to the above-mentioned example, but various deformation is possible for it based on the meaning of this invention. For example, although experimented in the purity of 1-kestose about 99.9% of thing, it cannot be overemphasized that the thing of more low purity is also usable.
[0030]
[Effect of the Invention] Whether it uses 1-kestose independently or uses together the viable cell preservative of this invention with other viable cell preservatives, it has the preservation effectiveness excellent also in the thing [any] case. The viable cell preservative of this invention has effectiveness not only in cryopreservation but in preservation at the low temperature of extent which is not frozen.
[0031] Since mass production method can do 1-kestose by microorganism industry, a viable cell preservative can be offered cheaply.

TECHNICAL FIELD

[Industrial Application] In case this invention saves a viable cell at a freezing condition or low temperature for a long period of time, it relates to the sperm of a required viable cell preservative especially the mammals, and fishes, and the viable cell preservative of an animal culture tissue.

PRIOR ART

[Description of the Prior Art] Although the viable cell has a living function and biological activity, and is various and there is utility value, under the conditions that temperature is high, change, a living function, and biological activity will fall thru/or disappear with time because of a metabolic turnover or

denaturation. Many researches are done till today, such as JP,60-29471,B which was considered that cryopreservation is suitable for the mothball of such a viable cell, for example, planned the having-two-incomes effectiveness of an intracellular frost damage protector and an extracellular frost damage protector, JP,63-216476,A which used methyl cellulose as a cryoprotective agent, and JP,2-422,A which used the betaine as a cryoprotective agent.

[0003] Generally, the following item is mentioned as conditions for the matter which show the frost damage prevention effectiveness over a viable cell.

** It is the neutral matter (neutral solute).

** It is the low-molecular matter (namely, matter with the high permeability over a cell).

** Have colligative property (it is easy to make colligative property, i.e., hydrogen bond, and the eutectic point is a low property).

[0004] ** The high concentration of the toxicity over a cell is also low.

A glycerol and DMSO (dimethylsulfoxide) are in the matter which fulfills these conditions and is now used widely. However, it is rare to use it independently about a glycerol, and the mixed liquor of a glycerol and sugar is widely used for the sperm cryopreservation diluent of an animal, for example, a cow.

[0005] It is raised that that one-time dilution is possible for the merit at a room temperature, a glycerol balancing time's being shortened, and the freezing velocity of the large range are applicable etc. Moreover, since the frost damage prevention effectiveness of a saccharide excels 5 and 6 **** (Pentose, Hexose) in 2 and 3 saccharide, the raffinose which are mainly three saccharides has been used conventionally.

EFFECT OF THE INVENTION

[Effect of the Invention] Whether it uses 1-kestose independently or uses together the viable cell preservative of this invention with other viable cell preservatives, it has the preservation effectiveness excellent also in the thing [any] case. The viable cell preservative of this invention has effectiveness not only in cryopreservation but in preservation at the low temperature of extent which is not frozen.

[0031] Since mass production method can do 1-kestose by microorganism industry, a viable cell preservative can be offered cheaply.

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] However, the chemosynthesis of a raffinose is impossible, and if it does not extract and refine, the vegetation, for example, the beat etc., etc. which exists naturally, it cannot come to hand. Since the amount of supply was restricted, it was expensive. Moreover, since DMSO was an organic solvent, actuation of washing in cold water etc. might be needed, generally handling was complicated and what changes to this was desired strongly.

[0007] These people acquired the knowledge of it having already applied for the approach of manufacturing 1-kestose of a high grade cheaply by using cane sugar as a raw material (Japanese Patent Application No. No. 224312 [two to]), and the preservation effectiveness of a viable cell being in this 1-kestose. Then, this invention aims at offering the viable cell preservative which used 1-kestose.

MEANS

[Means for Solving the Problem] In order to solve said trouble, let this invention be the viable cell preservative characterized by containing 1-kestose as an active principle. Moreover, let this invention be the viable cell preservative characterized by adjusting 1-kestose concentration to 0.1 - 20% (W/V).
[0009] Moreover, let this invention be the viable cell preservative characterized by using 1-kestose for preservation of the viable cell chosen from the sperm of mammalian, the sperm of fishes, or the animal culture tissue. 1-kestose is so-called kind of a fructo oligosaccharide, fructose carries out 1 molecular binding of the structure to the fructose part of the shoe cloth (GF) which a glucose (G) and fructose (F) combined by beta-2 and 1 association, and it is O-alpha-D-glucopyranosyl. -(1->2)- It is an O-beta-D-hula KUTOFU llano sill. -(1->2)- It is O-beta-D-FURAKUTO furanoside. The molecular formula is C18H32O16, and the molecular weight is 504. Next, the structure expression of 1-kestose is shown.
[0010]
[Formula 1]

[0011] Although the application of the conventional fructo oligosaccharide had the diuretic currently indicated by the BIFIZUSU activity currently indicated by JP,59-53834,B and JP,59-110621,A, all were the mixture of the mixture of a fructo oligosaccharide, i.e., 1 kestose, (GF2), nistose (GF3), and FURAKUTO furanosyl nistose (GF4). The manufacturing method of 1-kestose is indicated by JP,2-163093,A and JP,2-249493,A, and can be acquired by making the cell tosyl transferase which the Scopulariopsis BUREBI cow squirrel (Scopulariopsis brevicaulis) produces act on cane sugar. Moreover, these people have already applied for the approach of manufacturing 1-kestose of a high grade cheaply by using cane sugar as a raw material, as Japanese Patent Application No. No. 224312 [two to].
[0012] According to this producing method, moreover, 1-kestose is industrially obtained in large quantities by the high grade (99.9%) individually. This high grade 1-kestose acquires the knowledge of having the effectiveness which was excellent as a viable cell preservative, and came to complete this invention. Using 1-kestose used in this invention by the concentration suitable for the viable cell to freeze, the concentration is 0.1 - 20% (W/V) of within the limits. Moreover, 1-kestose of this invention can be used as a part or all of a presentation of a viable cell preservative. For example, in the cryopreservation of the sperm of mammalian, use can be carried out 0.1 to 0.5% (W/V) as an alternative of the raffinose which is one constituent in the existing viable cell preservative (a sugar content glycerol and albumen suspension).
[0013] Moreover, in the sperm and animal culture tissue of fishes, 1-kestose can be adjusted to 3 - 20% (W/V), and it can be independently used as a cryopreservation agent as it is. Although it has an example in below and this invention is explained to it, this is instantiation to the last and this invention is not limited to this.
[0014]
[Example 1] About the cryopreservation of the sperm of a cow, the viable cell preservative containing 1-kestose of this invention was used, and it experimented in the preservation effectiveness. The viable cell preservative which consists of a constituent containing the raffinose which is the viable cell preservative currently used from the former as contrast was used. About the viable cell preservative, the raffinose only replaced the thing of contrast and this invention with 1-kestose, and all other components are the same.
[0015] The presentation of the cryopreservation liquid used for the experiment is shown in the next table 1.
[0016]
[Table 1]

[0017] After the experiment conditions of the cryopreservation of the sperm of a cow are -196 degrees C and saved freezing temperature for six months, they were thawed and investigated motility of sperm. The judgment of the vital force of a sperm should observe the sperm under the microscope, should move what moves forward with sufficient vigor (+++), the thing (++) which moves forward, and the head (+), and made (+++) the figure (%) of motility of sperm. The result is shown in the next table 2.
[0018]
[Table 2]

[0019] According to Table 2, it turns out that the viable cell preservative containing 1-kestose of this invention shows effectiveness equivalent to the viable cell preservative containing the conventional raffinose.
[0020]
[Example 2] 1-kestose, the water solution of a raffinose, or the DMSO solution (what was dissolved in 25mM Tris-HCl and pH7.5) was 80microl Added to 20micro of sperms l of a cherry salmon (Oncorhynchus masou), and 60microM and a DMSO solution prepared each stock solution, as 1-kestose and a raffinose became 2% about the last concentration. In addition, 1-kestose used the thing of 99.9% of purity. Thus, it put on the hollow with a diameter of 5mm which made 50micro of each prepared stock solution l with the drill on dry ice, and it quick-froze and the frozen sperm pellet was created.
[0021] This sperm pellet was saved at -70 degrees C, it thawed at 4 degrees C two days and 51 days after, and maneuverability was investigated under the microscope. Maneuverability just behind **** (time amount from movement initiation to a halt) was set to 100. The result is shown in the next table 3.
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